Gonadotrophin-induced accumulation of 'interstitial fluid' in the rat testis

R. M. Sharpe

M.R.C. Unit of Reproductive Biology, 2 Forrest Road, Edinburgh EH1 2QW, U.K.

Summary. 'Interstitial fluid' containing high levels of testosterone (60–250 ng/ml) was recovered from the testes of rats, the amounts increasing with increase in age and testis weight. Injection of 170 i.u. hCG/kg resulted 20 h later in significant increases in interstitial fluid and its testosterone content (300–800 ng/ml). In immature rats this effect of hCG was dose-dependent and time-related and the accumulated fluid contained high levels of potassium and phosphate; levels of sodium, calcium and protein were similar to those in serum. At 20 h after injection of hCG, other testicular changes were (1) increased 'adhesiveness', (2) reduced in-vitro binding of ^{125}I -labelled hCG, and (3) an hCG-induced increase in the testis:blood ratio of hCG *in vivo*.

Introduction

Injection of male rats with human chorionic gonadotrophin (hCG) or luteinizing hormone (LH) results in a prolonged decrease in testicular LH (hCG) receptors (Hsueh, Dufau & Catt, 1976; Sharpe, 1976; Haour & Saez, 1977; Purvis, Torjesen, Haug & Hansson, 1977) and, in certain circumstances, a reduced capacity of the testis to secrete testosterone (Hsueh *et al.*, 1977; Sharpe, 1977a). In the immature rat, injection of hCG also results in a transient but substantial (20–35%) increase in testicular weight due to the accumulation of fluid (Sharpe, 1977b). The present paper describes this effect in more detail in rats of different ages, and attempts to relate 'interstitial fluid' accumulation to other changes induced by hCG treatment.

Materials and Methods

The animals were from our inbred strain of L/H rats and were housed as described previously (de Jong & Sharpe, 1977). At 22 days of age male rats were injected subcutaneously with 10, 1 or 0.1 i.u. hCG (Chorulon: Intervet) in 0.5 ml saline (9 g NaCl/l), and killed with ether 20 or 48 h later, when testicular 'interstitial fluid' was collected as described below. Similar procedures were followed when male rats aged 35, 50, 70 or 200 days were killed at 20 h after injection of hCG (170 i.u./kg) at a dose roughly equivalent to the 10 i.u. given to the 22-day-old rats. For all the groups equivalent numbers of saline-injected control rats were used.

Measurement of 'interstitial fluid'

The paired testes from individual rats were removed, cleaned of extraneous material, placed into pre-weighed 5-ml plastic serum vials and weighed. A small incision was then made in the caudal end of each testicular capsule, the testes were replaced in the vials and the interstitial fluid was allowed to collect in the bottom of the vial kept for 4 h at 4°C. The testes were then removed and the fluid remaining in the vials was weighed and stored at –20°C. When thawed, the

fluid was centrifuged for 5 min at 1500 g and 0.1 ml of the supernatant was removed and diluted with 0.01 M-phosphate-buffered saline (pH 7.5) containing 0.1% gelatin (Sigma). Testosterone was then measured by radioimmunoassay as described by Corker & Davidson (1978) except that samples were assayed directly, as interstitial fluid from hCG-treated rats had previously been shown to give comparable results ($N = 12$, $P > 0.1$ by paired t test) before and after extraction with 10 volumes of hexane:diethyl ether (4:1, v/v). Unextracted interstitial fluid from control and hCG-treated adult rats gave dose-response curves which were parallel to the standard curve ($P > 0.05$ in a test of non-parallelism by two-factor analysis of variance) over the range tested (0.1 to 0.01 μ l equivalents of interstitial fluid). In the present study all samples were assayed together at doses in the range 0.1 to 0.02 μ l, and the intra-assay coefficient of variation was $\pm 4\%$. Other details of the assay method and separation procedure were as given by Corker & Davidson (1978). The sensitivity of the assay was 120 pg/ml. Quantitative assessment of the concentration of various non-hormonal factors in interstitial fluid and serum from immature hCG-treated rats was kindly performed by Dr J. Seth using a computer-linked Technicon sequential autoanalyser.

Binding of ^{125}I -labelled hCG

After the above procedures, testes from control and hCG-treated rats were decapsulated and homogenized, and the in-vitro binding of ^{125}I -labelled hCG was determined as described previously (Sharpe, 1976) using 20 mg homogenate and 15 ng ^{125}I -labelled hCG/ml for incubation. The hCG (CR119; 11 600 i.u./mg) was labelled with ^{125}I by the method of Miyachi, Vaitukaitis, Nieschlag & Lipsett (1972) to a specific activity of 64 $\mu\text{Ci}/\mu\text{g}$. The in-vivo testicular binding of ^{125}I -labelled hCG was assessed as described previously (Sharpe, 1976) following injection of rats aged 25, 60 or 120 days with 10 ng ^{125}I -labelled hCG (0.12 i.u.) alone or together with 10 i.u. unlabelled hCG in 0.2 ml 0.01 M-phosphate-buffered saline.

Isolation of Leydig cells from the testes of saline- and hCG-treated immature rats was by the method of Moyle & Ramachandran (1973) using collagenase (Type 1; Sigma) at 0.25 mg/ml and 1 ml/testis; dispersion of testicular tissue was effected by incubation for 20 min at 37°C in a shaking water bath (100 cycles/min).

The significance of differences between means was assessed by Student's t test.

Results

Interstitial fluid accumulation

Compared to controls, injection of 22-day-old rats with 1 or 0.1 i.u. hCG had only minor effects on the recoverable amounts of interstitial fluid at 20 h after injection, whilst treatment with 10 i.u. hCG increased fluid levels 20-fold at this time (Table 1); at 48 h after treatment with these doses of hCG, interstitial fluid values were not different from those of controls (Table 1). Testis weight and recoverable interstitial fluid increased with increase of age in control rats (Table 2) and at each age injection of 170 i.u. hCG/kg significantly increased testis weight 20 h later; depending on age, between 56 and 99% of this change could be accounted for by increased amounts of interstitial fluid (Table 2).

Interstitial fluid composition

The concentration of testosterone in interstitial fluid from pubertal (50- and 70-day-old) control rats was 3–4 times higher than the levels in adult (200-day-old) rats, and a similar pattern was evident following injection of hCG, although this treatment significantly increased the fluid concentration of testosterone at each age (Table 2).

Table 1. Accumulation of interstitial fluid (mean $\pm$ s.d.) in the testes of 22-day-old rats (4/group) at 20 h and 48 h after injection of various doses of hCG

Dose of hCG injected (i.u.)	Time when killed (h)	'Interstitial fluid' (mg)
0 (control)	20	5.0 $\pm$ 6.6
10		107.5 $\pm$ 22.5***
1		11.5 $\pm$ 10.8
0.1		16.3 $\pm$ 6.2*
10	48	7.5 $\pm$ 6.2
1		5.0 $\pm$ 7.6
0.1		8.5 $\pm$ 6.5

Significantly different from control value,
* $P < 0.05$; *** $P < 0.001$.

Table 2. Testicular weight, 'interstitial fluid' and binding of ^{125}I -labelled hCG in rats of different ages at 20 h after injection of saline or 170 i.u. hCG/kg

Age (days)	Treatment	Paired testis weight (mg)	'Interstitial fluid' (mg/paired testes)	Testosterone in 'interstitial fluid' (ng/ml)	Binding of ^{125}I -labelled hCG (pg/20 mg)
35	Saline	802 $\pm$ 84	44 $\pm$ 7	—	99.2 $\pm$ 15.0
	hCG	953 $\pm$ 69*	193 $\pm$ 24***	384.4 $\pm$ 43.9	39.2 $\pm$ 5.0***
50	Saline	1966 $\pm$ 78	158 $\pm$ 24	245.5 $\pm$ 57.8	272.6 $\pm$ 42.2
	hCG	2131 $\pm$ 92*	250 $\pm$ 21**	767.5 $\pm$ 22.8***	127.4 $\pm$ 19.0***
70	Saline	2459 $\pm$ 96	192 $\pm$ 42	264.7 $\pm$ 101.7	425.7 $\pm$ 56.2
	hCG	2689 $\pm$ 76***	338 $\pm$ 42***	519.5 $\pm$ 125.7**	209.9 $\pm$ 81.6***
200	Saline	2898 $\pm$ 235	360 $\pm$ 61	67.1 $\pm$ 17.7	389.9 $\pm$ 72.1
	hCG	3235 $\pm$ 213*	581 $\pm$ 97***	332.0 $\pm$ 74.7***	176.5 $\pm$ 34.3***

Results are the mean $\pm$ s.d. of 4–7 animals in each group.

Significantly different from respective control value, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

The concentration of various non-hormonal factors in serum and interstitial fluid, collected 16 h after injection of 25-day-old rats with 10 i.u. hCG, is shown in Table 3. The protein content and concentrations of sodium and calcium in interstitial fluid were similar to those found in serum whereas the concentrations of potassium and phosphate ions were higher than those in serum.

Table 3. A comparative analysis of serum and 'interstitial fluid' from hCG-treated immature rats

	Serum	'Interstitial fluid'
Sodium (mmol/l)	141	140
Potassium (mmol/l)	5.7	14.7
Calcium (mmol/l)	2.56	2.85
Phosphate (mmol/l)	2.24	3.85
Total protein (mg/ml)	54	49
Albumin (mg/ml)	42	41
Urea (mmol/l)	5.0	5.4
Urate (mmol/l)	0.14	0.75
Alkaline phosphatase (units/l)	570	454
Aspartate aminotransferase (units/l)	190	1230

Analyses are based on a pool of material collected over a 30-min period from the testes of eight 25-day-old rats killed 16 h after injection of 10 i.u. hCG.

Text-fig. 1. Age-dependent changes in the uptake of ¹²⁵I-labelled hCG *in vivo* by rat testes (a) after injection of 10 ng ¹²⁵I-labelled hCG and (b) after injection of 10 ng ¹²⁵I-labelled hCG + 10 i.u. unlabelled hCG. Results are plotted as the ratio of c.p.m. radioactivity per mg testis divided by c.p.m. radioactivity per mg blood (T/B). The inset shows disappearance curves for ¹²⁵I-labelled hCG in blood. ●—●, 25-day-old rats; ○—○, 60-day-old rats; △—△, 120-day-old rats. Values are the mean $\pm$ s.d. for 3 animals at each point.

Binding of ¹²⁵I-labelled hCG

In control rats, *in-vitro* testicular binding of ¹²⁵I-labelled hCG increased 4-fold between 35 and 70 days of age when adult levels were reached, whilst injection of hCG reduced this binding by 50 to 60% at each age (Table 2).

In vivo, the ratio of radioactivity in the testes to that in the blood (T/B) after injection of 10 ng ¹²⁵I-labelled hCG, increased ($P < 0.01$) between 4 and 12 h in 25-day-old rats and between 4 and 20 h in 60- and 120-day-old rats and then declined gradually (Text-fig. 1a). In comparison, injection of 10 i.u. unlabelled hCG + 10 ng ¹²⁵I-labelled hCG (0.12 i.u.) resulted in a similar pattern of uptake but with a doubling ($P < 0.01$) of the T/B ratio of radioactivity at 12 and 20 h in all three age groups (Text-fig. 1b); this occurred despite the effective dilution of circulating levels of ¹²⁵I-labelled hCG by a factor of 1:80. Blood levels of ¹²⁵I-labelled hCG were highest at 4 h after injection and then declined with a half-life of approximately 12 h (25-day-old rats) or 20 h (60- and 120-day-old rats). In another experiment in which 25-day-old male rats were killed 16 h after injection of 10 i.u. unlabelled hCG + 10 ng ¹²⁵I-labelled hCG, the ratio of radioactivity in interstitial fluid to that in the blood was 1.13 ± 0.08 (mean $\pm$ s.d., $N = 5$) whereas in the same rats the testis tissue:blood ratio was significantly higher ($P < 0.001$) at 2.78 ± 0.51 .

'Adhesiveness'

The preparation of testicular homogenates for receptor-binding studies (Sharpe, 1976) involves resuspension of a sedimented pellet in buffer. This is normally achieved simply by vortexing for 5–10 sec but the pellet failed to resuspend when tissue was taken at 12, 16, 20 and 24 h from 21-day-old rats after injection ($N = 3$ or 4 at each time) of 10 i.u. hCG and only partly resuspended at 6 and 8 h after injection. Resuspension was normal by 30 h after injection.

The preparation of isolated Leydig cells using collagenase results in cell dispersion within 20 min when testes of immature control rats are used. In contrast the testes from 4 rats killed 20 h after injection of 10 i.u. hCG remained completely intact even after 45 min incubation with collagenase, and similarly at 20 h after injection of 4 i.u. hCG, minimal dispersion of the

testicular tissue was achieved. At 40 h after injection of 10 i.u. hCG normal testicular dispersion was achieved by 20 min of incubation with collagenase.

Discussion

Most reports concerning testicular fluid in the literature refer to rete testis fluid which is believed to be secreted by Sertoli cells into the lumen of the seminiferous tubules (see Waites & Setchell, 1969; Setchell, 1970). The testicular fluid described in the present paper is referred to as 'interstitial fluid' as this is presumably where it accumulates in the testis. Using techniques similar to those presently used, Pande, Chowdhury, Dasgupta, Chowdhury & Kar (1966) collected fluid from the untreated rat testis and considered it to represent testicular lymph, which normally fills the interstitial spaces of the rat testis (see Fawcett, Neaves & Flores, 1973). Although the composition of 'interstitial fluid' appears similar to that described for testicular lymph (Pande *et al.*, 1966; Setchell, 1970), it has a much higher concentration of potassium; this may be a consequence of cell damage (perhaps indicated by the high level of aspartate aminotransferase) or leakage of the ion from the seminiferous tubules, as the level of potassium found was similar to that reported for rete testis fluid (Setchell, 1970). However, in other respects, 'interstitial fluid' and rete testis fluid appear quite different. Firstly, the latter has very low levels of protein and phosphate in relation to serum (Setchell, 1970) whereas 'interstitial fluid' has a similar protein content and an elevated level of phosphate compared to serum. Secondly, whereas hCG stimulates the accumulation of 'interstitial fluid' it has been reported to have either no effect (Setchell, Duggan & Evans, 1973) or to reduce the flow rate of rete testis fluid at 12 to 24 h after injection (Laporte & Dacheux, 1977a, b). Thirdly, the concentration of testosterone in 'interstitial fluid' from rats of different ages treated with hCG was in the range 300–800 ng/ml which is considerably higher than the levels reported by Harris & Bartke (1974) for rete testis fluid in rats treated with hCG, but is similar to values reported in interstitial fluid obtained by micro-puncture of the rat testis (Comhaire & Vermeulen, 1976).

It has previously been shown (Sharpe, 1977b) that injection of immature rats with 10 i.u. hCG led to no increase in 'interstitial fluid' until after 6 h with levels peaking at 12–20 h and falling to control levels by 40 h after injection. The present data also show fluid accumulation at 20 h after injection of 170 i.u. hCG/kg and, in addition, demonstrate that at 12 to 20 h after injection of 10·12 i.u. hCG (10 i.u. unlabelled plus 0·12 i.u. ^{125}I -labelled hCG) there is a doubling of the testis/blood ratio of ^{125}I -labelled hCG compared to that found following injection of 0·12 i.u. ^{125}I -labelled hCG only. This change is due mainly to increased binding of ^{125}I -labelled hCG to testis tissue rather than to its accumulation in 'interstitial fluid' because the ratio of radio-activity in the latter to that in the blood was near to unity whilst the testis tissue:blood ratio was 2·78. This hCG-induced increase in the testicular binding of hCG has been reported previously for immature rats (Sharpe, 1976) but its precise cause is unknown. It seems most likely that it results from changes such as increased capillary wall permeability or increased testicular blood flow and the findings of Hartman, Millman & Stavorski (1950) would support this view. It may be this change which results in the accumulation of 'interstitial fluid', although the presently observed increase in 'adhesiveness' of homogenized testis tissue between 8 and 20 h after injection of immature rats with 10 i.u. hCG is suggestive of some other change occurring. The fact that, at 20 h after hCG injection, testis tissue failed to disperse with collagenase suggests that this change might be ionic, and the very high concentrations of potassium and phosphate in 'interstitial fluid' are perhaps significant in this respect.

At 20 h after injection of hCG, when interstitial fluid accumulation is near maximal, there are two other important effects of this hormone on the testis. Firstly, there is a reduction in the *in-vitro* testicular binding of ^{125}I -labelled hCG which is related, at least in part, to a loss of LH (hCG) receptors (Hsueh *et al.*, 1976, 1977; Sharpe, 1976) and secondly, there may be a 50%

decrease in the stimutable capacity of the testis to secrete testosterone (Hsueh *et al.*, 1977; Sharpe, 1977a). It is especially interesting that the latter effect only occurs (in the immature rat) with doses of 10 i.u. hCG or more, precisely the dose which causes major accumulation of 'interstitial fluid'. It is also relevant that in the cryptorchid adult rat, in which serum levels of LH are 3–4 times higher than normal, as much as 50% of testicular weight may be accounted for by 'interstitial fluid' containing high levels of testosterone (Sharpe, Kerr & de Kretser, 1978).

The hCG-induced accumulation of 'interstitial fluid' in the immature rat testis can be inhibited by cycloheximide, a treatment which also interferes with the induced loss of LH receptors (Sharpe, 1977b). This raises the possibility that these two effects of hCG may be dependent on the synthesis of the same protein(s). However, in contrast to the hCG-induced accumulation of 'interstitial fluid', LH receptor loss persists for long periods (Hsueh *et al.*, 1976; Sharpe, 1976), is not maximal until 2 days after treatment (Hsueh *et al.*, 1977) and occurs even with very small doses of hCG (Sharpe, 1976) which, as shown here, do not result in major accumulation of 'interstitial fluid'. It is possible that hCG-stimulation of the testis always results in the formation of 'interstitial fluid' and it is simply the balance between formation and clearance that determines whether this fluid accumulates in measurable quantities. It seems clear that a better understanding of the causes of 'interstitial fluid' accumulation will also increase our understanding of LH receptor regulation and associated changes in steroidogenic function as all three factors are, at least initially, determined by the same gonadotrophic stimulus.

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